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Lycopladine H, a novel alkaloid with fused-tetracyclic skeleton from *Lycopodium complanatum*

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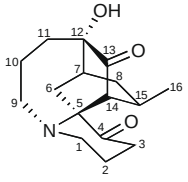
ABSTRACT

A novel *Lycopodium* alkaloid, lycopladine H (**1**), with a fused-tetracyclic ring system consisting of an azocane ring connected to a [2,2,2]-bicyclooctane ring and a 3-piperidone ring, was isolated from the club moss *Lycopodium complanatum*. The structure and relative stereochemistry of **1** were elucidated on the basis of spectroscopic data.

Club moss (Lycopodiaceae) is known to be a rich source of *Lycopodium* alkaloids[1] possessing unique heterocyclic ring systems such as $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$, which have attracted great interest from biogenetic,[2] synthetic,[3] and biological[4] points of view. In our continuing efforts to find biogenetically interesting and structurally unique *Lycopodium* alkaloids, we have isolated a number of new *Lycopodium* alkaloids[5] possessing complex fused-cyclic skeletons, which have attracted as challenging targets for total synthesis.[6] Further investigations of extracts of newly collected *Lycopodium complanatum* resulted in the isolation of a new *Lycopodium* alkaloid, lycopladine H (**1**), possessing a novel fused-tetracyclic ring system consisting of an azocane ring connected to a [2,2,2]-bicyclooctane ring and a 3-piperidone ring. In this Letter, we describe the isolation and structure elucidation of **1**.



1

The club moss *L. complanatum*[7] (2.33 kg) collected at Nayoro in Hokkaido was extracted with MeOH aq, and the extracts were partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, adjusted at pH 9 with satd Na_2CO_3 aq, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (*n*-hexane/EtOAc, 1:0→1:1, $CHCl_3$/MeOH, 1:0→1:1, and then $CHCl_3$/MeOH/H_2O, 6:4:1). The HMQC spectrum ($CDCl_3$) of the fraction eluted with *n*-hexane/EtOAc (4:1) showed a cross-peak[8] at δ_H 2.90/δ_C 55.0, which did not ascribe to any known *Lycopodium* alkaloids, indicating the existence of a new alkaloid. Therefore, the fraction was further purified by a silica gel column chromatography ($CHCl_3$/MeOH, 200:1) to afford lycopladine H (**1**, 0.00003% yield).[9]

Lycopladine H (**1**) {$[\alpha]_D^{22}$ −116 (*c* 0.34, $CHCl_3$)} showed the pseudomolecular ion peak at *m/z* 278 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{16}H_{23}NO_3$, was established by HRESIMS [*m/z* 278.1745, $(M+H)^+$, Δ −1.1 mmu]. IR absorptions implied the presence of hydroxy and keto carbonyl (3450 and 1720 cm^{-1}, respectively) functionalities. ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum revealed the existence of 16 carbons due to two carbonyl carbons, two sp^3 quaternary carbons, three sp^3 methines, eight sp^3 methylenes, and one methyl group. Among them, a signal for one sp^3 quaternary carbon (δ_c 78.5) was attributed to a carbon attached to a hydroxy group.

The gross structure of **1** was elucidated by analysis of 2D NMR data including the ^1H–^1H COSY, TOCSY, HMQC, and HMBC spectra in CD_3OD (Fig. 1). The ^1H–^1H COSY and TOCSY spectra of **1** disclosed three structural units **a** (C-1–C-3), **b** (C-6–C-8 and C-15–C-16), and **c** (C-9–C-11). Connectivities of C-1 (δ_C 48.7), C-5 (δ_C 70.3)[10], and C-9 (δ_C 53.0) through a nitrogen atom were revealed by HMBC correlations for H-1a (δ_H 3.35) to C-5 and C-9. An HMBC correlation for H_3-16 (δ_H 0.87) to C-14 (δ_C 56.1) suggested the connectivity between C-14 and C-15. HMBC cross-peaks of H-15 (δ_H 1.73) to C-5 and C-13 (δ_C 215.7) indicated the connectivity of

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Table 1
^{1}H and ^{13}C NMR data of lycopladine H (**1**) in CD_3OD at 300 K[a]

Position	δ_H	δ_C	HMBC
1a	3.35 (1H, ddd, 13.3, 10.1, 5.0 Hz)	48.7	3a, 3b, 9a, 9b
1b	2.67 (1H, br d, 13.3 Hz)		
2	1.98 (2H, m)	27.4	3a
3a	2.62 (1H, m)	37.4	1a
3b	2.41 (1H, br d, 15.0 Hz)		
4		207.6	3a, 3b, 14
5		70.3	1a, 6a, 6b, 7, 9a, 9b, 14, 15
6a	2.60 (1H, m)	26.0	8b, 14
6b	2.23 (1H, br d, 15.9 Hz)		
7	2.08 (1H, m)	40.3	6a, 8b, 11a, 11b
8a	1.74 (1H, m)	32.2	6a, 6b, 14, 15
8b	1.55 (1H, m)		
9a	3.25 (1H, ddd, 15.5, 8.0, 3.3 Hz)	53.0	1a, 11a, 11b
9b	2.73 (1H, ddd, 15.5, 7.9, 4.0 Hz)		
10a	1.80 (1H, m)	26.5	11a, 11b
10b	1.67 (1H, m)		
11a	2.01 (1H, m)	39.8	9a, 9b
11b	1.88 (1H, ddd, 14.2, 7.8, 4.3 Hz)		
12		78.5	6a, 8a, 11a, 11b, 14
13		215.7	7, 11b, 14, 15
14	2.96 (1H, s)	56.1	15, 16
15	1.73 (1H, m)	28.5	8a, 8b, 16
16	0.87 (3H, d, 6.5 Hz)	21.4	8b, 14, 15

[a] ^{1}H and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively.

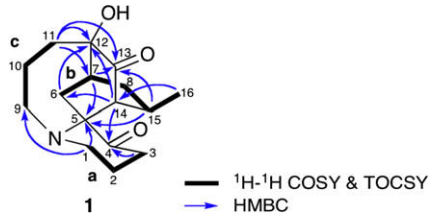


Figure 1. Selected 2D NMR correlations for lycopladine H (**1**).

C-5, C-13, and C-15 via C-14. Connectivities of C-4 (δ_C 207.6), C-6 (δ_C 26.0), and C-14 through C-5 were elucidated by HMBC correlations for H-14 (δ_H 2.96) to C-4 and C-6, and H-7 and H-11b to C-13. The connectivity of C-7 (δ_C 40.3) and C-11 (δ_C 39.8) through C-12 (δ_C 78.5) was implied by HMBC correlations for H-6a (δ_H 2.60) to C-12, and H_2-11 (δ_H 2.01 and 1.88) to C-7 and C-12. An HMBC correlation for H-14 to C-12 revealed the connectivity of C-12 and C-13. An HMBC cross-peak of H_2-3 to C-4 suggested that unit **a** constituted a part of the 3-piperidone ring (C-1–C-5 and N-1). Thus, the gross structure of lycopladine H was elucidated to be **1** (Fig. 1).

The phase-sensitive NOESY spectrum of **1** showed cross-peaks as shown in computer-generated 3D drawing (Fig. 2). The chair form of the 3-piperidone ring was revealed from NOESY correlations for H-14/H-1a and H-14/H-3a. NOESY cross-peaks of H-6b/H-9a and H-6b/H-10a suggested that C-9 was in an axial position of the 3-piperidone ring. NOESY correlations for H-6a/H-8a and H-8b/H-16 indicated an equatorial position of C-16 in the cyclohexane ring (C-5–C-8 and C-14–C-15). Thus, the relative stereochemistry of lycopladine H was assigned as **1** (Fig. 2).

Plausible biogenetic path for lycopladine H (**1**) is proposed in Scheme 1. Recently, we proposed that lycopladine A[5b] could be derived from an intermediate possessing phlegmarane skeleton through a hypothetical precursor X (route a). Lycopladine H (**1**) may also be generated from the precursor X by nucleophilic addition of the nitrogen atom to C-9 and Mannich-type addition of C-14 to C-5 (route b).

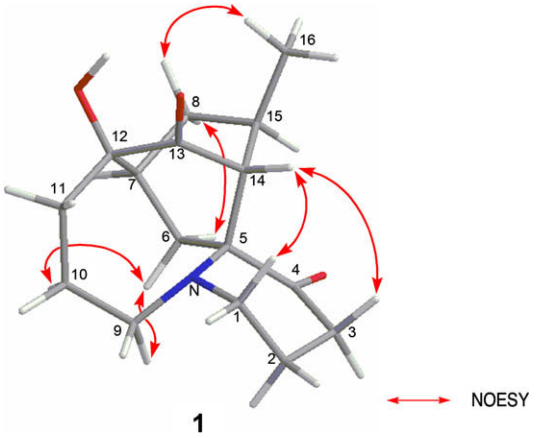
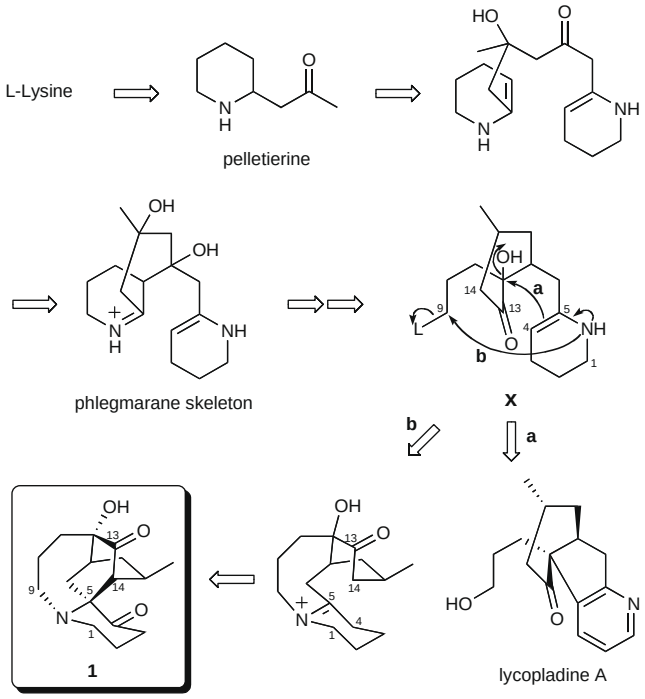


Figure 2. Selected NOESY correlations and relative stereochemistry for lycopladine H (**1**).



Scheme 1. Plausible biogenetic path for lycopladine H (**1**).

Lycopladine H (**1**) is an unprecedented $C_{16}N$-type *Lycopodium* alkaloid possessing a novel fused-tetracyclic ring system consisting of an azocane ring (C-9–C-14, C-5, and N-1) fused to a [2,2,2]-bicyclooctane ring and a 3-piperidone ring. Lycopladine H (**1**) did not show cytotoxicity against L1210 murine leukemia and KB human epidermoid carcinoma cells (IC_{50} >10 μg/ml) in vitro.

Acknowledgments

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7. The voucher specimen has been deposited in the herbarium of Hokkaido University.
8. This cross-peak corresponded to H-14/C-14 of **1**.
9. Lycopladine H (**1**): colorless amorphous solid; $[\alpha]_D^{22}$ −116 (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3450, 2920, 1720 cm^{-1}, ESIMS *m/z* 278 $(M+H)^+$; HRESIMS *m/z* 278.1745 [$(M+H)^+$ calcd for $C_{16}H_{24}NO_3$, 278.1756].
10. A spiro carbon connected to a nitrogen atom and a carbonyl carbon of compound **15** was observed at relatively low field (δ_C 69.3) in ^{13}C NMR spectrum: Kaden, S.; Reissig, H.-U. *Org. Lett.* **2006**, *8*, 4763–4766.